EXHIBIT

15

DEFENDANTS' MOTION TO EXCLUDE THE TESTIMONY OF DR. CHRISTOPHER TEAF

05-CV-0329 GKF-PJC

IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA, et al.,	)	
	)	
Plaintiffs,	)	
	)	
vs.	)	**05-CV-0329 GKF-SAJ**
	)	
TYSON FOODS, INC., et al.,	)	
	)	
Defendants.	)	
	)	

EXPERT REPORT
of
HERBERT L. DuPONT, M.D.

October 14, 2008

IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

EXPERT REPORT OF HERBERT L. DuPONT, MD

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Qualifications and Experience

My name is Herbert L. DuPont. I am over 18 years of age and am competent to testify. All opinions presented in this statement reflect personal knowledge based on information and data that I have reviewed in this case. All opinions provided in this affidavit are given to a reasonable degree of scientific certainty.

Education: I received a Bachelor's degree with a major in chemistry from Ohio Wesleyan University in 1961 and an MD from Emory University School of Medicine and carried out internal medicine residency at the University of Minnesota Medical Center and infectious diseases fellowship at the University of Maryland. I have served as an infectious disease epidemiologist (person who studies epidemics of infectious diseases in human populations) with the U.S. Centers for Disease Control and Prevention (CDC) and was asked to study infectious diseases outbreaks in the U.S. and abroad.

Current Positions: I am currently Director of the Center for Infectious Diseases and Professor of Epidemiology at the University of Texas – Houston School of Public Health and have taught the principles of intestinal infections, diarrheal illness and water-borne and food-borne disease outbreaks and evaluation to public health and medical students at our school for more than thirty years. Also, I am Chief of Internal Medicine at St. Luke's Episcopal Hospital, a 700-bed university hospital responsible for training doctors in internal medicine including infectious diseases, nephrology, hematology, endocrinology, gastroenterology, as well as eight other medical specialties.

Research Work and Publications: I have worked for approximately thirty years performing research in the field of enteric (intestinal) infections and infectious diseases in the U.S. and abroad. I have authored or co-authored more than 570 scientific papers and 19 textbooks, most of which have been in the field of infectious and intestinal infectious diseases. I direct research programs dealing with diarrhea in the United States and abroad, currently working on three continents. Much of the current information on dose of enteric (intestinal) organisms causing diarrhea in humans come from volunteer challenge studies performed by me. I am considered an international expert in infectious diseases, diarrhea and intestinal infection.

Involvement with Federal Agencies: I have had recurrent involvement with U.S. Public Health Service, after formal training beginning when I served as an Epidemic Intelligence Service Officer with the CDC. During the Carter administration, I was asked to serve as one of four medical consultants to develop a research program between Israel, Egypt and the United States, from 1989 through 1994, as part of the Jimmy Carter-Anwar Sadat-Menachem Begin Camp David Accords. I served on the Vaccine and Biological Products Committee of the U.S. Food and Drug Administration, February 1989-January 1993 and remain to the present time a Special Government Employee and Advisory Committee Member to the FDA. I served on the Board of Scientific Counselors for the CDC in Atlanta, from 1992 through 1996 and served on the National Institutes of Health (NIH) Blue Ribbon Panel on Bioterrorism and its Implications For Biomedical Research, commissioned just after September 11, 2001.

Editorial Boards: I served as Associate Editor of the American Journal of Epidemiology and the Journal of Infectious Diseases, and currently serve as the Deputy Editor of the Journal of Travel Medicine. I am

currently on the following Editorial Boards of major medical journals: Clinical Infectious Diseases, Infectious Diseases in Clinical Practice, The Journal of Infection, Infectious Disease News, and Gastroenterology & Hepatology

Honors and Recognition: I was recognized for teaching excellence from the University of Texas Medical School fifteen times including receipt of the school's most distinguished clinical teacher award and the outstanding teaching award from the medical school alumni. I received the Bronze Medaille D'Honneur (Bronze Medal of Honor) from the French government, April 25, 1993 for leadership in travel medicine. The International Society of Travel Medicine bestowed on me their Medal of Honor in 1995, in gratitude for leadership as first society president and for work in the development of the Journal of Travel Medicine. The Society has given two Medals of Honor in its more than 20 years of existence. I received an Honorary Doctorate from the University of Zurich at a formal ceremony in Switzerland, April 24, 2004, in recognition of achievements in areas of understanding how Escherichia coli produces diarrhea and furthering knowledge of the causes and mechanisms of infectious intestinal infections. I received the Distinguished Achievement Citation from Ohio Wesleyan University, the university's highest honor for alumni, for contributions to scientific research, teaching, clinical practice and service to the global community. I received the Maxwell Finland Award for Scientific Achievement for outstanding contributions to understanding of infectious diseases and public health from the National Foundation for Infectious Diseases in Washington, DC, March 21, 2007. This is the top national award in infectious diseases. May 15, 2008, I was awarded Mastership in the American College of Physicians (ACP) at a formal Convocation, Washington, DC. On April 4, 2007 I was asked to deliver a national address - the 15th annual James H. Steele, DVM Lecture on Foodborne Disease which was published in the journal Clinical Infectious Diseases. I am a member of Who's Who in America and Who's Who in the World. I have been listed in the Best Doctors in America and America's Top Doctors for infectious disease each year of the two publications (1992-2008 and 2001-2008, respectively).

Assignment: For the current lawsuit I have been retained by the defendants to assess and respond to the state's expert testimony and to provide my assessment of the poultry industry and the allegation that it may have contributed to contamination of the Illinois River Watershed. My work has included addressing the allegation that poultry litter has created a public health risk. I have reviewed a large volume of material provided by the state in this suit and the various Expert Reports and depositions of the State's consultants: Darren L. Brown, PH, Lowell Caneday, PhD, Bernard Engel, PhD, P.E, J. Berton Fisher, PhD, Valerie J. Harwood, PhD, Gordon V. Johnson, Ph.D, Todd W. King, PE, BCEE, Robert S. Lawrence, MD, Dr. R. Jan Stevenson, Dr. C. Robert Taylor and Christopher M. Teaf, PhD. I have also reviewed the material these consultants considered in forming their opinions.

Compensation: For my work I am compensated at a rate of $450 per hour for any work provided including testifying.

Approach Taken in This Report

In the following comments, I deal with key issues relating to waterborne infectious diseases in the United States and in the state of Oklahoma among persons living near and recreating in the Illinois River Watershed. For my report I direct my comments to eleven relevant areas and then

provide a brief summary of my conclusions. I include detailed references of the points made providing scientific support for my comments. I have treated this as a scientific document and provided documentation and support for my opinions. I have previously prepared an affidavit and provided testimony in this matter. I hereby incorporate my prior affidavit and testimony the same as if fully set forth herein.

1. **Bacterial Markers of Fecal Contamination of Water "Fecal Indicator Bacteria" (FIB) is an Evolving System of Monitoring Safety of Water Used for Recreation and for Drinking and a Distinction Should be Made Between Inexpensive Screening for Water Quality Versus Incriminating a Single Source or Industry Responsible for Contamination**

Fecal pollution of water sources can come from wastewater treatment facilities, septic tanks, domestic animals, wild animals including water fowl and pets. Since enteric disease-producing pathogens can come from a variety of human or animal sources and disease control and prevention depends upon knowing the origin and source or reservoir of disease-producing microbes, an emphasis in water quality measurements has moved from finding coliforms of possible fecal origin to more accurate markers of pollution. The recent past emphasis has been on defining *E. coli*, a non-specific fecal organism found in all warm-blooded animals. The current and future emphasis is and will continue to be identifying disease-causing pathogens in water. Water pollution of human origin is of the greatest public health concern since human feces is more likely to contain human-specific microbes than is animal feces. Within animal species the range of pathogens destined for humans is fairly narrow with a number of specific disease-causing organisms showing predictable animal or human reservoirs.

Historically "intestinal" bacteria have been sought in water to indicate that possible fecal contamination has occurred. The indicator bacteria traditionally relied upon include total coliforms (or "*E. coli*-like" organisms), fecal coliforms (defined by biologic properties), *Enterococcus* and *E. coli*. The latter two organisms are found in the intestines of all warm blooded animals. There are many limitations of relying on these fecal markers in water as a means to show disease risk with recommendations for water monitoring changing with the development of modern molecular methods of microbiological detection.

In 1986 the U.S. EPA recommended that *E. coli* be used as the indicator of fecal contamination in recreational waters. The standard was set at a geometric mean concentration of 126 colonies per 100 mL of water, which was estimated to be correlated with gastrointestinal illness rate of approximately 8 people per 1,000 swimmers. This was established in freshwater beaches on Lake Erie in Pennsylvania and on Keystone Lake near Tulsa, Oklahoma. Swimming was strictly defined as activity that resulted in all upper body openings being exposed to the water. The beaches had different levels of fecal indicator bacteria. After 8 to 10 days the swimmers and non-swimmers were interviewed with regard to symptoms of gastrointestinal or respiratory illness. *E. coli* and enterococci showed some correlation with illness in these areas heavily used by humans for bathing and swimming but fecal coliforms did not show a relationship with gastrointestinal illness in the swimmers. An important point about these standards is that popular beaches were studied where heavy human use of the water and nearby human sewage discharges assured the presence of bacteria of human origin including human disease-causing pathogens. The standards

were not set in more remote areas where human use and living was minimal to give the worst case scenario.

Limitation of Fecal Indicator Bacteria to Determine Water Quality

While it is understandable that the water industry must have an inexpensive and useful means of screening water for microbial quality, in a lawsuit aimed at a single industry, indicator bacteria have no value in determining an industry contribution to water quality. In the following I offer six points about the limited value of indicator bacteria as a means of monitoring water quality and determining contribution of a single industry to microbial contamination.

Fecal indicator bacteria (FIB) are not considered pathogens

Indicator bacteria may be more predictive of water quality when they are not found, making it unlikely that other disease-causing microbes associated with fecal pollution will be present. When indicator bacteria are detected, pathogens may or may not be present.

It is important for lay persons to understand *E. coli* as both good and bad bacteria. The normal *E. coli* that live in our intestines are good for us. They take up room and inhibit the subsequent growth of more hostile organisms and thus directly protect us. Also, *E. coli* and other normal microbial flora of our intestinal tract produce vitamins that are important to our health. Other bacteria that in the laboratory resemble our normal good *E. coli* produce toxins or can invade the lining of our intestine and produce human illness. These are called pathogenic bacteria and can produce diarrhea and other symptoms. When I evaluate water samples for *E. coli* in the water in the absence of an epidemic, I assume the *E. coli* are not disease-producing. This relates to the agreed upon point that indicator should not be considered pathogenic. The exception that indicator bacteria never cause human disease is seen with some of the pathogenic strains of *E. coli* that come from human feces (enterotoxigenic *E. coli* - ETEC) or from cattle manure (*E. coli* O157:H7 and other serotypes of Shiga toxin producing *E. coli* – STEC, such as *E. coli* O111). Poultry is not a recognized source of ETEC or STEC. Additionally, the state has not shown the presence of ETEC or STEC in poultry litter or in the IRW.

Bacteria grow to much higher levels in food, which is a culture medium, than in water, which inhibits growth of bacteria. If we used the same system of monitoring of food as water which makes perfect sense, we would never eat fresh fruits and vegetables. In published data from our laboratory, we have found that pre-washed and bagged lettuce obtained from grocery stores in Houston contain an average of 30,000 coliforms per gram of weight. In Europe by federal laws, cheese for consumption may contain up to 10,000 *E. coli* per gram (1). The same principles dealing with risk of intestinal infections and fecal contamination (indicator bacteria) exist for food and water. When comparing these levels of "fecal indicator bacteria" in food with water, it should be remembered that the number of bacteria measured in water is per 100 mL. For comparison, the number of indicator bacteria in Houston lettuce per 100 mL would be 3,000,000 (3 million) per 100 g (equivalent to 100 mL as measured in water) and the number of acceptable indicator bacteria in acceptable cheese for consumption in Europe would be 1,000,000 (1 million) per 100 g. These numbers in food regularly consumed by Oklahoma residents need to be compared to the low number of indicator bacteria found in the IRW. Oklahoma residents will

find much lower numbers of indicator bacteria in the waters of the IRW than in their local grocery store. The organisms have the same significance in food and water.

One of the errors made by state consultants is that *Enterococcus* from water is a pathogen for humans. The organism is seen essentially only in patients in a hospital setting who acquire the organism after being given broad spectrum antibiotics (2), generally in an intensive care unit. *Enterococcus* is not considered by infectious disease physicians to be a waterborne pathogen of patients.

> Indicator bacteria as a means of evaluating water disease risk, lack of sensitivity and specificity

Fecal indicator bacteria have been used to determine safety of water for more than 100 years. The argument supporting their use is that if there are no feces in the water, there will be no disease-causing organisms of intestinal origin, whether the source of the organisms be from animals or from humans. Modern state laboratories using measurements of fecal indicator bacteria for water quality are moving away from the assay of coliform and fecal coliform measurements, which are poor indicators of fecal pollution, as indicated by the University of California Agriculture and Natural Resources Department, (Good Agricultural Practices, http://groups.ucanr.org/UC_GAPs/Eliminate_Fecal_Coliforms/) and the U.S. EPA (3). These agencies suggest focusing on *E. coli* as the indicator organism that often reflects a fecal origin from animals or humans (4), while developing methods to actually measure disease-producing pathogens. Data from the IRW and other bodies of water should not use coliforms or fecal coliforms for any predictive value for water quality. These parameters no longer can be considered useful.

Concerning source of contamination, laboratory methods currently employed in testing of water do not differentiate between good or bad *E. coli* or *E. coli* of human versus animal origin, thus limiting the value of *E. coli*-based assays (5). To confirm that water is unsafe for humans, either as drinking water or recreational water, it is more relevant to assay for virulent human pathogens in the water than looking for indicator bacteria including *E. coli* (6). The food industry has learned this long ago and our country does not routinely culture the more than $40 billion of food imported from international countries for coliform bacteria or *E. coli*. If they employed the same standards for food that are used for water, our grocery stores would contain very few items.

Presence of fecal markers does not indicate that disease-producing bacteria are present in the water (7). A study looking for presence of indicator organisms in six wastewater reclamation facilities failed to show that any one indicator was predictive of the presence of disease-causing microbes (6), which agrees with other studies in which fecal indicator bacteria did not correlate with presence in water of pathogenic microbes (8). In all these studies, the gold standard was finding pathogens in the water, not indicator bacteria.

> Current measurements of indicator bacteria provide no information about source of contamination, which is critical to establishing causation and when looking for contribution of a single source or industry and for developing control measures

Fecal indicators do not tell the source of the contamination whether it be from animal species, human beings or the environment (9). Research laboratories are developing methods to help determine origin of intestinal bacteria. See later discussion of microbial source tracking (MST).

> When looking at the contribution of a single industry or animal source, laboratory methods of water quality should focus on detection of pathogens in water, particularly those pathogens known to be associated with the industry of interest

The most important laboratory approach in determining whether water poses a risk of human illness is direct identification of disease-causing microbes in the water (10). Research groups are actively working on this area (see area number 6, below for an extensive discussion of this topic).

> Bacterial markers (fecal indicator bacteria) currently being assayed in rivers and lakes often come from the environment without directly representing either an animal or a human fecal source

The bacterial markers may not directly reflect organisms of fecal origin. Studies have shown that *E. coli* can persist in water sources throughout the year in adjacent soil, sediments, bank seeps (11) and algae (12). Finding persistent foci of *E. coli* and other fecal indicator bacteria alive and propagating in soil (13) and multiplying in wetlands (14) limits the value of fecal indicator bacteria as markers of fecal contamination or as warnings that disease-causing microbes are present.

> The laboratories performing biologic marker studies often provide inaccurate results

The various laboratories performing these assays frequently make errors in laboratory work providing inaccurate data (15) underscoring the need for targeted epidemiologic studies to determine the presence of a water associated public health problem.

2. The Levels of Fecal Indicator Bacteria in the IRW Approximate Levels Seen With Many Water Sources in Oklahoma and the United States and Human Disease Rates in the Watershed Approximate those Seen in Other Regions

The presence in water of low numbers of bacteria of possible fecal origin does not predict a body of water will be associated with increased rates of human disease (16). The key is to show that an organism of water origin is actually causing human illness in a watershed area. This is done though epidemiological study which is critical to providing evidence that one or more waterborne pathogens are producing illness in people (10). Epidemiologic investigations are methodologically sound and are essential to learning whether a problem exists and what disease-producing pathogens are responsible for human illness (17). Such epidemiological studies have allowed public health authorities to focus on the pathogen(s) of interest as has been done in previous waterborne outbreaks in the United States including outbreaks from: recreational water of Crater Lake caused by enterotoxigenic *E. coli* (ETEC) from a human source (18); drinking water in Rome, New York caused by *Giardia* due to contamination by beavers and other lower mammals (19); drinking water associated *Cryptosporidium*-induced illness in Milwaukee (20)

from cattle; and drinking water in Aspen, Colorado due to *Giardia* (21) from lower mammals in the wild. If epidemiologic studies had not been performed these outbreaks would likely have remained undetected. Also, none of these outbreaks came from poultry operations or from poultry.

Finding that isolated spots in the IRW have occasionally failed to meet the EPA 1972 Clean Water Act standards does not mean that it is unsafe for human recreation or that excessive illness will result by human exposure. Approximately 35% of rivers and 45% of lakes in the U.S. do not meet the 1972 Clean Water Act standards to be swimmable or fishable, thus are considered impaired (USEPA. The quality of our Nation's waters; EPA-841-S-00-001; US Environmental Protection Agency, Office of Water: Washington, DC, 2000). The only way that reduced human safety on the part of a body of water can be determined is by epidemiologic study of human illness. The only suitable indirect method to determine lack of safety of a water body is finding well-defined and fully-virulent enteric pathogens in the water.

The Plaintiff consultants estimate based on indicator bacteria and the number of person using the Illinois River and its tributaries, that 8 per 1,000 people (0.8%) develop illness each year because of the contaminated water. Considering the number of persons using the water provided by Dr. Caneday, the consultants estimate that 1,200 illnesses occur annually because of the water quality. In my opinion, there is no scientific basis for this estimate. The human health risks from water depend upon a number of factors including presence of pathogens in the water, source of indicator bacteria (animals versus humans) and use of the water. Concerning use of the water, floating or boating, where ingestion of water is unlikely, is not associated with risk for waterborne disease. Drinking water from household wells is of greater concern if pathogens are present in the water. Of perhaps greater importance than this estimate of 1,200 illnesses from water exposure, there is absolutely no reason to point to the poultry industry as the important source of water contamination. If excessive waterborne disease is occurring in the IRW, I believe it would most likely reflect water pollution from local human sources (e.g. septic tanks), with water pollution from cattle or wild animals both being more likely than pollution from poultry sources.

The Health Commissioner from Oklahoma, Dr. Michael Crutcher was not concerned with the number of cases of enteric infections in his state. If I had been the health commissioner, I too would have come to this conclusion based on the number of cases of illness identified in the counties adjacent to the IRW. Dr. Crutcher did not suggest that epidemiologic studies be performed. I reviewed the data on reported human cases of *Campylobacter* and *Salmonella* diarrhea in the counties adjacent to the IRW and found their rates comparable with most of the counties of the United States outside the IRW. There is absolutely no data to suggest that an enteric microbial health risk has existed or exists in the IRW.

To determine that water microbes are actually capable of causing human disease, it is necessary to perform epidemiological studies looking at human cases and determining human risk (22). The arguments of the Oklahoma state consultants about cases of human enteric illness along the IRW are curious. On one hand they state that most illness cases are not detected by normal surveillance so there must be many undetected cases of disease occurring related to the local water sources. On the other hand, they argue that focused epidemiologic study is not needed to

determine actual risk, since no clustering of cases has been documented. Without any scientific justification, they claim to know there is a problem without clinical or epidemiologic evidence, instead use only faulty logic. When epidemiologic studies are not recommended or performed, health authorities are satisfied with rates of illness found with their normal surveillance of disease. In these settings, as in the IRW, health authorities have enough information about disease rates in their population and need no further study.

3. In the Absence of Heavy Water Contamination and Large Community Illness Outbreaks, Disease-Producing Microbes in Water Sources Are Not Present or Present in Low Concentrations, Showing Low Rates of Infectivity with Organisms of Non-Poultry Sources Predominating

Water supports the growth of relatively low levels of bacteria unless contaminated with raw human sewage. Microbes can survive for variable time periods in water but it is a relatively hostile environment for microbial viability and growth. For this reason, cultures of water looking for bacteria, indicator bacteria or pathogens, must determine presence of microbes in a volume of water, usually 100 mL. In contrast, food is a microbial growth facilitating media and bacterial cultures are performed per one gram of food (equivalent to 1 mL or 100^{th} of the amount of water cultured routinely). The organisms of concern for poultry contamination are *Campylobacter* and *Salmonella*. There are no other human disease-causing microbes found importantly in poultry or poultry feces (see later comments). For that reason, I will concentrate on these two disease-producing microbes in further discussion.

The most important variable in developing human illness from contaminated water is presence of human excreta associated with full water-body contact and submersion of the head with ingestion of recreational water (23).

4. Food is the Major Source of Human Enteric (Intestinal) Infections in the United States and in Oklahoma with Water Producing Much Lower Rates of Human Illness

The CDC estimates that there are 76 million cases of foodborne illness each year in the United States. If we assume that there are 298,000,000 persons in the U.S. and that there are 3,600,000 persons in Oklahoma, we would expect to have approximately 918,118 cases of foodborne illness in the state each year. The consultants for the state estimate that the water of the IRW causes 1,200 cases of illness each year. While I am not convinced by their figures, it is useful to compare 1,200 with nearly one million to see where the state should focus their attention in efforts to improve the health of Oklahoma residents.

The EPA estimates that in the U.S. drinking water causes approximately 8.5% of the cases of acute gastroenteritis seen in the U.S. translating to 16. 4 million cases per year (24). In drinking water outbreaks, four enteropathogens have been most commonly implicated, *Cryptosporidium*, *Giardia*, noroviruses and *Shigella*. For each of these organisms, illness can be produced in humans after exposure to low doses (less than 100 organisms, perhaps a single organism) and human waste from adjacent septic tanks most commonly explains ground water contamination (25).

A less important source of human illness is exposure to recreational water. The most important organisms causing waterborne illness from recreational waters in the U.S. are Cryptosporidum, *Shigella*, *Giardia*, noroviruses, Shiga toxin-producing *E. coli* including O157:H7 and enterotoxigenic *E. coli* (ETEC) (26-30). These organisms gain entrance to recreational water primarily from: 1) cattle, for *Cryptosporidium*, *E. coli* O157:H7 and *Salmonella*; 2) humans for *Shigella*, noroviruses and ETEC; and 3) lower mammals such as beavers for *Giardia* (31).

Poultry is not considered a source of waterborne microbes. A majority of gastroenteritis outbreaks from recreational water come from swimming pools or fountains not from recreational lakes or rivers (32). Wading and paddling pools are the most important source of *E. coli* 0157:H7 where the very low dose pathogens reach the water from non-toilet-trained and infected toddlers (33).

When epidemiologic studies are combined with environmental investigation in recreational waters of the U.S., illness outbreaks are known to be caused by contamination of waters by lower mammals, water fowl, and human sewage-contaminated recreational water and ground water (34). Once more, poultry is not known to be an important source of waterborne enteric pathogens.

5. Water is Primarily a Source of Low-Dose Pathogens with Higher-Dose Pathogens Being Transmitted by Food in Which Bacterial Multiplication to Infectious Levels is More Apt to Occur

Pathogens can be classified as low-dose (<100 viable organisms regularly produce illness in healthy persons); intermediate-dose (500-100,000 viable microbes) and high-dose (>100,000). The low-dose organisms are *Shigella*, *Giardia*, *Cryptosporidium*, *E. coli* O157:H7 and other Shiga toxin-producing *E. coli*, rotavirus (for infants) and noroviruses. The intermediate dose organisms are *Campylobacter* and *Salmonella* and the high-dose organisms are enterotoxigenic and enteroaggregative *E. coli* and Vibrio cholera, the cause of cholera. Since water tends to inhibit the growth of bacteria, the low dose pathogens are the most important organisms in water-borne infections. When the concentrations of pathogens in water are high, particularly when important contamination has occurred with human sewage, all classes of organisms may be transmitted through water including the high-dose organisms, as is seen in cholera endemic areas.

One important epidemiologic clue that an organism is a low-dose pathogen is that the organism is frequently spread from infected persons to susceptible individuals through personal contact, so called, "person-to-person" spread. Also, low-dose pathogens are the ones seen causing recurrent infections among infants and toddlers attending day care settings where there is a low density of fecal organisms in the environment. In a mathematical assessment of dose of *Campylobacter* based on human feeding experiments, the dose producing the highest illness-to-infection ratio was found at an intermediate dose of 90,000 organisms (35). While in milk with its acid-buffering capacity, a lower dose (possibly as low as 500 living organisms) may rarely be achieved (36), which is not relevant for a water source of illness. *Campylobacter* and *Salmonella* are not normally spread person-to-person and are not considered important problems in day care

centers because of the moderate dose required to produce disease. These infections are spread by food and less commonly in water where multiplication to required infectious levels first occurs.

Dose needed to cause illness in persons has been established by human challenge (feeding) studies. While it is logical to think that immunocompromised persons or young infants can be infected with lower organism inoculums, there are no data to prove that *Salmonella* or *Campylobacter* cause illness in this group of people with low inoculum size. Publications that give doses of organisms needed to cause infection and illness that are not based on scientific study base their ideas on pure speculation without clinical or epidemiologic evidence. I reviewed the information given in the US FDA Bad Bug Book and looked for evidence that 15-20 *Salmonella* could cause illness depending on age, health or host or strain differences. The book provides no data to support their dose estimate. If such a dose produced illness in humans it would be very unusual based on feeding experiments and disease epidemiology. The Bad Bug Book does not mention *Campylobacter* as a low dose organism, defined as producing disease in an inoculum size less than 100 bacteria.

The more important concept than dose when immunocompromised persons become infected with one of these organisms is severity of disease when it does occur. All of us in the field of infectious diseases caring for the many patients who are immunocompromised including those with acquired immune deficiency syndrome (AIDS), widespread cancer or the elderly with other comorbid diseases, know the predictable infectious diseases seen in these populations. The predictable pathogens include Pseudomonas or *Klebsiella* for cancer patients, Mycobacterium avium intracellulare and cytomegalovirus for AIDS patients and *Pneumococcus* and *Clostridium difficile* for elderly persons with comorbidity. These infections are well known to physicians who care for these patients such as infectious disease physicians. While immunocompromised persons are exposed daily to foodborne enteric agents including *Salmonella* and *Campylobacter*, these organisms aren't more commonly seen in these persons than in healthy individuals. As an infectious disease consultant when I see an immunocompromised person with diarrhea, I worry more about *C. difficile*, *Listeria*, a parasite or a herpes virus than *Salmonella* or *Campylobacter* even though I know they ingest these organisms weekly. Immunocompromised persons don't commonly acquire *Salmonella* or *Campylobacter* from other family members when they experience one of the infections, again suggesting that they aren't more susceptible to the organisms. If immunocompromised persons had an inordinately increased rate of *Salmonella* and *Campylobacter*, infectious disease physicians like me would see each day cases of these forms of diarrhea. This just doesn't happen.

Another mistake I've seen in the testimony of Dr. Harwood, in her deposition of July 18, 2008. She indicated that Guillain Barre paralysis occurs in less than 5% of patients with *Campylobacter* infection. While the causes of Guillain Barre Syndrome are characteristically unknown, constituting an idiopathic disorder, this complication only rarely is seen following *Campylobacter* infection, seen in approximately 1.17/1,000 person-years or <2/10,000 during the 2-months after *Campylobacter* diarrhea (37).

6. Recovery of Disease-Producing Microbes from Water – Where the Field is Moving in Determining Safety of Water and Source of a Potentially Waterborne Illness

Water is not a friendly environment for the growth of microbes. With water there is a built in safety factor which helps to explain the relatively low risk of acquiring intestinal illness from swimming in recreational water or when well water is consumed. Water dilutes the organisms present and a number of factors detailed here help keep the microbial counts low. One of the major factors in keeping the level of microbial growth in water at very low and safe levels is solar disinfection from the sun. Solar action lowers the counts of pathogens as has been shown for *Shigella* and the cholera bacteria in water (38). *Campylobacter* strains do not survive well in water. In sunlight-exposed waters *Campylobacter* is rapidly killed due to photo-oxidative damage (39), with survival in strong natural sunlight of only 20 minutes (40). In groundwater the *Campylobacter* die-off is 2.5 to 13 times faster than for the indicator bacterium, *E. coli* (41), showing the disconnect between the organisms.

As mentioned recurrently above, finding disease-causing microbes in the water is of far greater value in defining the role of water in causing human illness than is measuring fecal indicator bacteria. The methods required for recovery of pathogens differ from recovery procedures for food because of low bacterial counts in water. Conventional culture methods developed for high-inoculum food or stool are less accurate in finding waterborne pathogens. Methods have been developed, however, to directly detect bacterial pathogens in water and should be the focus of future water monitoring programs because of the poor correlation between indicator bacteria and pathogens in water sources (8).

While *Campylobacter* and *Salmonella* strains have been found to be stressed in the hostile environment of water, they can be recovered and identified from water sources. Some researchers have indicated these bacteria are viable but nonculturable by conventional laboratory methods (42, 43). This is not a correct concept. A better explanation is that most of the organisms are killed in an aqueous environment with recovery (resuscitation) occurring only for the small number of living and culturable bacteria (44). The state's consultants are incorrect in saying that pathogens cannot be recovered from water so there is no reason to look for them. If the state is implicating the poultry industry in allegedly making the IRW unsafe for humans, they must show that poultry-associated pathogens are in the water. These pathogens can be detected in water by modified culture methods and by non-culture molecular methods. Successful methods for culturing *Campylobacter* from water sources include the use of:

1) non-selective agar and enrichment broth maintained at reduced temperature (45);
2) enrichment using selective media (46, 47);
3) culturing water in fertilized chicken eggs (48);
4) passage of water samples in mice (49); and
5) concentrating bacteria by passing water through filters (50, 51).

When filtering water for *Campylobacter*, the water can be passed through a filter with pore size, 0.45 microns) (52, 53), or processed by ultra-filtration (54) or by employing membrane absorption-elution techniques (55).

Salmonella can be identified in water by PCR-hybridization methods (56), by magnetic capture hybridization combined with PCR or real-time PCR (57). PCR methods have been developed for

detection of *Campylobacter* and *Salmonella* in food (58) and the methods have been adapted to wetlands and water (59).

None of the information I was provided on this issue explained why the plaintiff experts weren't asked to attempt recovery of *Campylobacter* or *Salmonella* from the IRW. The methods are available as cited above.

7. *Salmonella* and *Campylobacter* are the Only Enteric (Intestinal) Pathogens Affecting Humans that Can Be Traced to Poultry; Both Organisms Can Come From Other Animals Including People

Campylobacter diarrhea in humans is associated with chicken consumption in between 25% to 50% of cases (60). For *Salmonella* infection, poultry, eggs and other animals, including cold-blooded animals are important sources of the organism. Most of the other pathogens causing human illness do not come from poultry because of a species barrier. Because of species specificity, organisms from humans are able to attach to receptors in the human intestine, can produce a human-specific inflammatory response or can invade the human bowel wall. Many organisms of animal origin, while possessing some of the virulence properties seen also in human pathogens, may not be able to infect a human host because of human lack of receptors of organism attachment. I offer some examples of species specificity for enteric pathogens. While *Cryptosporidium* is a human pathogen from cattle. This species is classified as *Cryptosporidum parvum*. The parasite in poultry is *Cryptosporidum avium*, adapted to infection in poultry, but is not able to infect other mammals or humans (61). Similarly, enterotoxigenic *E. coli* (ETEC) is a human pathogen causing waterborne outbreaks when the source of contamination is human sewage. Animal strains of ETEC, important in scours in the natural animal host, cannot stick to the human intestine and cause illness (62). *Giardia* from some animals (e.g. lower mammals such as beavers) can infect people and nearly all species of animals can be infected with the parasite. Many strains are not pathogenic for humans.

Consultants for Oklahoma have indicated that poultry represent a reservoir for *E. coli* O157:H7 infection in humans. There is no scientific evidence for this. The CDC in their website on the sources of this infection indicate: "the major source of *E. coli* O:157 is ground beef. Other foods and beverages are unpasteurized milk and juice, sprouts, lettuce and salami." Cattle are the source of *E. coli* O157for most human infections and cattle are frequently colonized by this important pathogen of humans regardless of whether they are grass-fed or lot-feed (63). Waterborne transmission of *E. coli* O157 strains occurs through swimming in contaminated lakes, pools, or drinking inadequately chlorinated water due to contamination from humans, often non-toilet-trained infants in wading pools. The organism is easily transmitted from person to person and has been difficult to control in child day-care centers." (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_t.htm)

The *E. coli* O157 strains that have been isolated from poultry and are found on the farm in general often lack disease-producing capability (64). Poultry-associated strains, while typed as O157, may not belong to the fully virulent O157:H7 variety, important as a pathogen in humans (65). There is no microbiologic or epidemiologic evidence to implicate poultry as a source of

human infection for any *E. coli* diarrhea-pathogens, including *E. coli* O157 and other Shiga toxin producing *E. coli* including *E. coli* O111.

The CDC estimates that 80% of *Campylobacter* illnesses in the U.S. come from food and that 95% of *Salmonella* illnesses come from food in the U.S. (66). This relates to the higher inoculum requirements and need first for organism multiplication in food before human illness to occur. Non-food risk factors for *Campylobacter* infection are foreign travel, receipt of antibiotics with alteration of intestinal flora, contact with home pets and consumption of unpasteurized milk (60). Non-food risk factors for *Salmonella* are limited and include international travel, exposure to amphibians (frogs and newts), baby chickens at Easter time or household pets fed natural pet treats and cleaning aquaria in sinks (60). Water is not considered an important source for either organism where multiplication to infectious levels does not occur.

8. Antimicrobial Resistance for *Salmonella*, *Campylobacter* and normal Intestinal *E. coli* Flora – Role of Poultry Versus Human Use of Antibiotics

Campylobacter resistance to the fluoroquinolone class of antibiotics (ciprofloxacin or cipro-related drugs) became a problem in the U.S. after the release of fluoroquinolones for animal and human use in the 1990s. During these years, the human use of this class of drugs became more widespread than animal use. This class of drugs has become the routine treatment of urinary tract infections, respiratory tract infections and bacterial diarrhea including travelers' diarrhea. The FDA suspended all fluoroquinolone use in poultry production as of September 12, 2005. The class of drugs is still available for livestock. The FDA ban on fluroquinolones in poultry did not lead to reduced resistance among *Campylobacter* in poultry products (67) suggesting that human use of the drugs and international travel were the dominant factors in the development of resistance (60). Fortunately we are able to treat *Campylobacter* infections in humans with readily available antibiotics including erythromycin and azithromycin (68). In the case of *Salmonella* infection, antibiotics are not used for cases of gastroenteritis (69). For septicemic *Salmonella* infection (blood poisoning) we have excellent antibiotics to manage the infection.

There is no evidence that antibiotic resistance spreads to the general population from poultry. A consultant for the state of Oklahoma warned that antibiotic use in the poultry industry would stimulate the development of a widespread pool of antibiotic resistant genes (plasmids) that would be spread to humans making infections resistant to normally effective antibiotics. There is a selective advantage to persons for this to happen only if the human is taking an antibiotic and this is the public health concern. We do need to improve the human use of antibiotics to prevent the problem discussed by the consultant. Looking at *E. coli* that colonize the human intestine for development of resistance, neither poultry contact or nor poultry consumption predicted the development of resistance, while foreign travel did (70). In relevant studies the percentage of persons carrying antibiotic-resistant *E. coli* was not larger in groups of meat-eating people compared with vegetarians providing additional data that a pool of antibiotic resistance from animals or poultry was not importantly being spread to people (71, 72).

9. Selected Extra-intestinal Infections of Humans and Water Exposure Discussed by State Consultants

Staphylococcus and Streptococcus are the two most important organisms causing soft tissue infection in humans (e.g. boils, impetigo or cellulitis). Both are found in the respiratory tract of humans. One third of persons are nasal carriers of Staphylococcus (73) and in these persons, the organism is spread from the nose where found in high numbers to other parts of the body producing infection when cuts or scratches occur. Streptococcus is also found in the respiratory tract of humans and is spread between people by the airborne route causing pharyngitis (sore throats). Poultry does not represent a known reservoir for human infection for either of these bacteria. While limited spread of the organisms has been seen on pig farms in Europe (74), and Staphylococcus can be grown from pigs, cattle, horses and pet dogs (75, 76), these animals are not established sources of the bacterium for humans and many of these animals including pets appear to have acquired the organism from their human contacts, rather than vice versa. There is no evidence that Staphylococcal and Streptococcal infections have been facilitated by poultry operations. Most of us in infectious diseases know the source of Staphylococcus and Streptococcus is nearly always from humans.

There are no standards for water safety for persons with cuts or scratches. Also, the relationship of water exposure to soft tissue infection in general remains an unstudied area. The organism important in soft tissue infection, *Staphylococcus* and *Streptococcus* show a human reservoir (source) with low counts found in water.

The two major types of ear infections are otitis externa (external ear infection) and otitis media (middle ear infection). The ear infection showing an important relationship to water recreation and swimming is otitis externa. Here the moisture in the outer ear from prolonged or intermittent submersion of the head in the recreational water, including swimming pools, produces an environment conducive to bacterial growth (77). The most common cause of otitis externa is *Pseudomonas* that grows in all water environments (78). *Staphylococcus* also can cause otitis externa. Poultry are not a source of *Pseudomonas* that is found in any moist environment and are not a recognized source of *Staphylococcus* causing waterborne infections.

The major eye infection associated with recreational water exposure (pools, rivers or lakes) is conjunctivitis or pink eye. This is caused by strains of adenovirus (79) spread to susceptible persons from infected people. The *Pneumococcus* bacterium from people also can cause pink eye. Poultry are not the source of adenoviruses or *Pneumococcus* linked to human conjunctivitis.

10. Microbial Source Tracking (MST) – Finding Origin of Pathogens Present in Water

Research groups are attempting to deal with the limitation of antiquated water quality monitoring looking at fecal indicator bacteria by employing various microbial source tracking (MST) methods (80). Molecular methods include immunoassay, nucleic acid methods such as polymerase chain reaction (PCR) and nucleic acid sequence based amplification and microarrays. An important line of research in this field has focused on detection of definable microbes (bacteria or viruses) found only in one specific animal population or humans, employing traditional microbiologic methods, phenotypic characterization or modifications of the PCR technology. Bacteria known to come from humans may be used to look for human pollution (81), which is of the highest risk for human illness. Bifidobacterial species have been used as a marker of human pollution. Some research groups have provided evidence that using

this biomarker may be useful (82) while others have not (83). *Enterococcus faecium* is a second such human bacterial marker (84). A third organism identified with human fecal pollution of recreational and surface water is human-specific *Bacteroides* spp. (85). Viruses that infect human bacteria, called coliphages can be used to track human specific microbes. This approach may allow the differentiation of fecal pollution of human origin from animal sources (86, 87).

One type of laboratory research in the area of MST has utilized modifications of the polymerase chain reaction (PCR) (88-90). Amplifications of regions of bacteria and gene frequencies can detect small numbers of bacteria in water, identify pathogens (91) and provide evidence that certain animal species are the source of origin of bacteria.

In using these methods, it is not appropriate to focus on only one source in attempting to determine the various potential sources of microbes in a water body such as the IRW. That is a biased approach that will complicate interpretation and erroneously implicate only one potential source, while not ruling out others. Groups that look for multiple sources have provided valuable information about various potential sources of contamination. Two studies are cited here to show how multiple sources might be studied providing useful information. In one of the studies, a modified PCR method demonstrated that cattle, geese, deer and humans all contributed to the *E. coli* found in streams under study (92). A second study demonstrated the importance of livestock (cattle, horses and sheep) to fecal contamination of regional streams (93). A third study implicated both humans and geese in the contamination of a major watershed (94). A common theme in many studies looking at recreational waters for source of fecal markers has been the finding that wildlife and adjacent human populations represent the most important sources of local water contamination (95-98). This is what is likely to be found in the IRW with adequate scientific study.

While nucleic acid technologies are versatile, there are two major problems with this methodology. First, PCR based methods cannot differentiate between living and dead organisms, with the latter having no health implications. Secondly, PCR based methods are ultrasensitive. In PCR based studies, DNA sequences from a few molecules are amplified to tens of millions of copies of the target DNA to test. An organism of interest can then be identified with only a few strains, dead or alive, being present in the water. This methodology does not allow a determination of concentration or amount of a microbe in water. It only shows presence or absence of the tracer organisms.

There are three flaws to Dr. Harwood's research each casting doubt on the significance of her findings:

1) Dr. Harwood developed a non-specific marker that may be found in avian populations and other sources. She did not develop specific molecular microbial markers appropriate for the broad range of animal species found in the IRW including cattle, wildlife (waterfowl and lower land mammals) and people. Not having results of studies with these biomarkers we cannot say anything about the relative importance of the various potential sources. It is obvious that Dr. Harwood was not asked to tackle the broader scientific question of source of contamination. Apparently she was asked to set up a "straw man", the poultry, for failure.

2) The molecular methods employed by Dr. Harwood as mentioned above are ultrasensitive. She amplified DNA from poultry litter or water samples, turning a few copies into many millions. To graphically illustrate this, if a handful of the bacteria she had identified had been dispersed in the IRW, it likely would have resulted in a positive test casting doubt on the public health significance of the research. The tests are so sensitive that they have no implications as to level of contamination.
3) Dr. Harwood found no illness-causing organisms in the various IRW sources or poultry litter and didn't develop molecular methods to look for these. It would have been easy for her to do this if asked. If all the living *Brevibacterium avium* in Oklahoma were introduced into the IRW, there is no reason to believe that one person would develop an illness as a result.

For the reasons stated, Dr. Harwood provided no direct or indirect evidence that poultry were contributing more fecal indicator bacteria to the IRW than cattle, waterfowl, lower mammals or people. She furthermore did not provide evidence that poultry contributed to the fecal indicator pool. Finally and most importantly, Dr. Harwood failed to provide any data that poultry had introduced or were introducing into the IRW, disease-causing microbes. It is disappointing she wasn't asked by her employers to develop experiments to determine if animals (any species) or people were introducing pathogenic microbes into the IRW. If she had not found genetic material of poultry origin on land where poultry litter was applied, I would have been surprised.

11. Hormones in the water of the IRW

When I make comments on this topic I am doing it as a Board Certified Doctor of Internal Medicine. Endocrinology and the medical aspects of hormones are part of my training and specialization. I have reviewed the Oklahoma state sampling data on hormones and feel qualified to comment on the significance of their findings. There are two major problems with the state's argument. The state has found nanogram concentrations of hormones in the water. A woman taking birth control pills ingests each day a million times this level. Women normally excrete into the environment large quantities of the measured hormones. The levels found in the IRW do not appear to me to be medically important. Secondly, the state has performed no studies to determine source of the hormones. In my opinion, the most likely sources of the hormones being measured in the IRW are cattle and humans. Once again, the state of Oklahoma is ascribing all water-related problems to the poultry industry without suitable study. I have reviewed FDA documents about this topic and find that they are interested in studying the area and are calling for more research both about the medical implications of hormones in water and in identifying the important source of the hormones found. They appropriately are seeking to put this issue into perspective and haven't indicated that poultry is the source of a problem. The websites for their requests for research are as follows: http://es.epa.gov/ncer/rfa/2006/2006_star_cafos.html, http://es.epa.gov/ncer/publications/workshop/08_20_07_cafo.html, http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.rfa/rfa_id/435

12. Conclusion

The case made by the state of Oklahoma about an alleged health risk within the IRW from the poultry industry is flawed for a number of reasons. While I have focused above on numerous problems with their case, in my concluding comments I comment on three fundamental flaws.

First, the state failed to perform the needed epidemiologic investigations to determine if an enteric infectious disease problem existed in the IRW. The health commissioner for the state of Oklahoma wasn't sufficiently worried about this based on rates of illness obtained during normal state disease surveillance, in my opinion, he shouldn't have been concerned. The state has provided absolutely no epidemiologic or clinical evidence that waterborne disease or diseases are occurring with unexpected or unacceptable levels in the region.

Second, the state's consultants attempted to find pathogens in the IRW but failed to find them. As a result, they hypothesized that pathogens were stressed, nonculturable but viable and stopped there. This is not a modern or scientific approach to assessment of a waterborne health threat. There are many studies showing that recovery of pathogens from recreational and drinking water is feasible and potentially important (see above). At a minimum, the state should have performed a thorough study looking for *Salmonella* and *Campylobacter* in drinking water and in recreational water in the IRW.

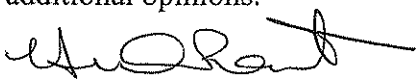
Third, the state has focused only on poultry as the potential source of environmental contamination in the IRW. They made a non-scientific decision to pursue the poultry industry ignoring all other sources of environmental contamination. Cattle are known to harbor and excrete into local environments bacterial pathogens destined to cause human illness, including *E. coli* O157:H7 and other Shiga toxin producing *E. coli* including O111 and O26 strains, *Salmonella* and *Cryptosporidium*. Wildlife regularly add to water sources human pathogens including *Giardia*, *Salmonella* and *Campylobacter*. Previous studies cited above have indicated that the three most important sources of contamination of water in the United States are people, cattle and wildlife. In reviewing the literature, I found no published studies providing evidence that poultry was a source of water contamination for human illness anywhere in the country.

Based on reasonable probability, I believe that in the IRW, humans are contributing importantly to contamination of drinking and recreational water. I base this statement on two lines of evidence. First, humans lead the list for water contamination in other settings referenced above. Secondly a report prepared on the Illinois River Basin of Oklahoma in 1999 provides evidence of an important local public health problem of human sanitation in the IRW. This report indicated that there were 27,000 septic systems in the 3 main Oklahoma counties of the IRW (Comprehensive Basin Management Plan for the Illinois River Basin in Oklahoma, prepared by Shanon Haraughty, Water Quality Division, Oklahoma Conservation Commission, May 1999). It furthermore indicates that a previous study found that only 25% of the on-site waste disposal systems met state requirements. The inadequacies found ranged from insufficient lateral lines, lack of sufficient septic tanks, direct disposal of grey (contaminated) water to streams ditches or land surfaces, and improperly located tanks and lateral lines. Extrapolation to the whole watershed suggests the potential for 75% of rural households to have substandard systems.

The court is being asked to assume poultry are the sole source of IRW contamination and to assume the region has excessive preventable infectious enteric disease related to the handling of

poultry litter. The efforts put forth in this lawsuit could have appropriately served the state of Oklahoma by a comprehensive approach to determining if a problem of water contamination existed and if so, where the contamination originated. Assuming that a problem exists in Oklahoma, which isn't clear to me, a comprehensive approach could lead to corrective measures and improved public health. A comprehensive and balanced study along with a corrective plan focusing on scientific findings is the only sure way to improve the public health from enteric pathogens in IRW. Such a program looking at all the various potential contributions to enteric disease in the state, would be welcome by the poultry industry and could be helpful to improving the health of residents of Oklahoma.

I look forward to evaluating further information and evidence on this topic and to providing additional opinions.



Herbert L. DuPont, MD
October 14, 2008

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